THE DETERMINATION OF ALKALOIDS BY EXCHANGE OF IONS

BY A. JINDRA AND J. POHORSKY

From the Institute of Pharmaceutical Chemistry, Charles University, Prague

Received November 15, 1950

IN a previous note[1] we indicated the general lines upon which ion exchange can be applied to the determination of alkaloids and we now give practical details for carrying out analyses, with instructions for standard methods for certain drugs and their preparations.

GENERAL

(i) *Assembly of the Apparatus.* This consists of a glass tube with its upper end conically widened and having a narrow outlet tube fused into the lower end. For the micromethod the dimensions are: total length 21 cm., diameter 0·8 cm., diameter of upper end 1·7 cm., length of broadened part 2 cm., length of chromatographic tube 14 cm., length of outlet tube 5 cm., diameter of outlet tube 5 mm. A rubber bung fitted into the top of the tube, carries a short delivery tube which is closed by means of a short piece of rubber tubing and a pinchcock.

The Amberlite IR-4B resin is ground under water in a porcelain mortar and transferred to a beaker with more water. The fine particles which remain suspended are poured off and the residue is washed with distilled water. Any further suspended particles are again discarded and the residue is left in the water for at least 48 hours. The resin is then ready for packing into the chromatographic tube and may be stored under water without undergoing change for a considerable time. The size of the resin grains varies between 0·8 mm. and 0·05 mm., the majority being about 0·2 mm.

(ii) *Titration Procedure.* A sufficiently sensitive electron potentiometer (Radiometer, type PHM 3H of Danish manufacture was used), automatic Schellbach's 2-ml. or 10-ml. microburettes with their tips drawn out to a fine capillary, a small water-turbine for stirring the titration solution and the following electrodes are required.

(a) A quinhydrone electrode made by adding solid quinhydrone to the titration solution; about 0·1 gr. was necessary because of the increased solubility in alcohol. The solution must be vigorously shaken for 1 minute before the actual measurement is made by dipping the platinum foil into the titration solution.

(b) A glass electrode type G/100, made as a supplement to Radiometer PHM 3 (non-shaded). Other shaded types were not available.

(c) Antimony electrode; a small antimony rod, cleaned with glass paper. There is always sufficient antimony oxide on its surface.

(d) A saturated calomel electrode.

(iii) *Solutions and Solvents Used.* Distilled water free from carbon dioxide and stored in well-closed containers, ethanol (90 per cent.), solu-

tion of crystalline sodium carbonate (4 per cent.), and 0·01N hydrochloric acid prepared by dilution of 0·1N acid with carbon dioxide-free water are required.

(iv) *Preparation of the Ion-exchange Column.* The tube is fixed vertically and the lower end is closed with a wad of cotton wool. The prepared resin is added while a steady flow of distilled water is maintained to prevent the formation of air bubbles. A second wad of cotton wool is placed on top of the column to keep it firmly packed. For the micro-determination the height of the column of resin is 2·5 cm. (about 1·8 g. of moist resin) and for the semi-micro-method it is 3·5 cm. high (about 2·5 g. of moist resin).

The resin is first regenerated by passing through it a quantity of 4 per cent. solution of sodium carbonate according to the length of the column (5 ml. is sufficient for the micro-method). The column is then washed with distilled water until the washing shows no reaction with phenolphthalein (about 70 or 80 ml. is required). For the semi-micro-method 10 ml. carbonate solution and 100 to 130 ml. of water water are necessary. To maintain the column ready for use a layer of water should be kept above it.

(v) *Standard micro-method.* About 5 mg. of alkaloidal salt is weighed with an accuracy of 0·01 mg. and dissolved in a 20-ml. conical flask in 5 ml. of ethanol (90 per cent.). The water in the column is then run off and replaced by 5ml. of hot ethanol (90 per cent.). Care must be taken in running off the water not to disturb the resin by causing air to be sucked in through the outlet tube. The pinchcock should therefore be loosened as the stopper is removed. As the ethanol leaves the tube the heated alkaloidal solution is added all at once and is collected in a titration beaker. The speed of flow should be about 60 to 70 drops per minute. The beaker in which the alkaloidal solution was prepared is then rinsed with 4-, 3- and 3-ml. quantities of hot ethanol which afterwards are passed through the column. These wash-solutions are manipulated so as to rinse the inside walls of the tube. The column is then washed with several ml. of distilled water and regenerated for further use. It may be used several times before it needs to be remade. In this case the resin is reground with a quantity of fresh material, the smaller particles being removed once again by flotation in distilled water. The alcoholic solution of alkaloid is then diluted with 35 ml. of boiled carbon dioxide-free water and electrometrically titrated from a 2-ml. microburette against 0·01N hydrochloric acid.

(vi) *Standard Semi-micro-method.* 10 to 30 mg. of alkaloidal salt, accurately weighed, is dissolved in 10 ml. of 90 per cent. ethanol. The column is washed with 10, 5 and 5 ml. of alcohol as in the micro-method. Before titration the eluate is diluted with 45 ml. of boiled carbon dioxide-free water and titrated against 0·01N hydrochloric acid contained in a 10-ml. microburette. The results vary within $\pm$ 1 per cent. of those obtained by other methods given in various pharmacopœias.

Assay of Some Drugs and Galenicals

Cinchona Bark. Approximately 80 mg. of powdered bark was accurately weighed, 1 ml. of water and 3 drops of 10 per cent. sulphuric acid were added, and the mixture heated in a water-bath for a short-time. After cooling, 10 g. of ether and 2 ml. of 5 per cent. ammonia were added and the entire mixture was vigorously shaken for 15 minutes. When the ethereal layer became clear, 7 g. of the ether (equivalent to 70 per cent. of the bark used) was carefully weighed into a small conical flask and evaporated to dryness on a water-bath. 1 ml. of 0·2 per cent. sulphuric acid and 5 ml. of ethanol (90 per cent.) were added and the resulting solution was treated as described in the standard methods and then passed through the ion-exchange column. The eluate was diluted with 20 ml. of water and titrated against 0·01N hydrochloric acid.

Liquid Extract of Cinchona. About 0·12 g. of extract was accurately weighed and dissolved in 5 ml. of water. 15 g. of ether and 1 ml. of 10 per cent. ammonia were added and the solution was vigorously shaken for about 10 minutes. When the ether layer had separated, 10·5 g. equivalent to 70 per cent. of the extract used was weighed off into a 20-ml. conical flask. It was then evaporated to dryness on a water-bath and the residue was dissolved in 1 ml. of 2 per cent. sulphuric acid and 5 ml. of ethanol (90 per cent.). The resulting solution was passed through the ion-exchange column as previously described, and the eluate was diluted with 20 ml. of water and titrated against 0·01N acid.

Dry Extract of Cinchona. About 0·04 g. of extract was dissolved in 5 ml. of water acidified with 2 or 3 drops of 10 per cent. sulphuric acid and was heated on a water-bath for 5 minutes. When cool 15 g. of ether and 1 ml. of 10 per cent. ammonia were added and the solution was treated as described under liquid extract of cinchona commencing "when the ether layer has separated." In this case, however, the eluate was diluted with 50 ml. of water.

TABLE I

Determination of the alkaloids of cinchona by ion-exchange (with antimony electrode)

	Experiment No.	Amount used g.	Volume of acid used ml.	Alkaloidal Content per cent.	Alkaloidal content by Czechoslovak Pharmacopœia I method per cent.
Bark	1	0·0874	1·23	6·18	6·20
	2	0·0777	1·10	6·22	
	3	0·0765	1·08	6·20	
	4	0·0813	1·17	6·32	
Liquid extract ...	1	0·1184	1·24	4·60	4·40
	2	0·1148	1·20	4·59	
	3	0·1112	1·17	4·61	
	4	0·1198	1·25	4·58	
Dry extract	1	0·0453	1·68	16·28	16·29
	2	0·0448	1·66	16·27	
	3	0·0366	1·36	16·33	
	4	0·0383	1·42	16·28	

1 ml. of 0·01N hydrochloric acid is equivalent to 0·003092 g. of alkaloids. Factor for acid used = 0·9939.

Ipecacuanha Root. To approximately 0·3 g., accurately weighed, of powdered root, 10 g. of ether, 1 ml. of 5 per cent. ammonia were added and shaken vigorously for 15 minutes. When separated, 7 g. of the ethereal layer was carefully weighed into a 20-ml. conical flask and evaporated to dryness. 1 ml. of 0·2 per cent. sulphuric acid and 5 ml. of ethanol (90 per cent.) were added to the residue and the resulting solution was passed through the ion-exchange column. The eluate was diluted with 20 ml. of water and titrated as before.

Tincture of Ipecacuanha. 2·5 to 3·0 g. of tincture was accurately weighed and evaporated on a water-bath until it weighed 0·6 to 0·7 g. After cooling 5 ml. of water, 10 g. of ether and 0·5 ml. of 10 per cent. ammonia were added and the mixture was shaken vigorously for 15 minutes. When separated, 7 g. of the ethereal layer was weighed off and treated as described under Ipecacuanha root.

TABLE II

DETERMINATION OF THE ALKALOIDS OF IPECACUANHA BY ION-EXCHANGE (ANTIMONY ELECTRODE)

	Experiment No.	Amount used g.	Volume of acid used ml.	Alkaloidal Content per cent.	Alkaloidal content by Czechoslovak Pharmacopœia I method per cent.
Root	1	0·2732	1·72	2·15	2·06
	2	0·3018	1·95	2·20	
	3	0·2925	1·84	2·14	
	4	0·2961	1·90	2·18	
Tincture	1	2·6976	1·20	0·152	0·153
	2	2·6151	1·15	0·150	
	3	2·8210	1·33	0·161	
	4	2·9820	1·36	0·156	

1 ml. of 0·01N hydrochloric acid is equivalent to 0·002402 g. of alkaloids. Factor for acid used = 0·9939.

Nux Vomica Seeds. Approximately 0·3 g. of powdered seeds, accurately weighed, was shaken vigorously for 15 minutes with 5 g. of chloroform, 15 g. of ether and 1 ml. of 10 per cent. ammonia. When the ether-chloroform layer had separated it was run off into 5 ml. of water and vigorously shaken for half a minute. After separation 14 g. of ether-chloroform layer was carefully weighed and evaporated almost to dryness. 1 ml. of ethanol (90 per cent.) was then added and the solution was completely evaporated. To the dry residue, 3 drops of 2 per cent. sulphuric acid and 10 ml. of ethanol (90 per cent.) were added and this solution was passed through the ion exchange column. The eluate was diluted with 25 ml. of water and titrated against 0·01N acid.

Tincture of Nux Vomica. 2 to 2·5 g. of tincture was accurately weighed and evaporated on a water-bath until the weight was 0·4 to 0·6 g. When cool 5 g. of chloroform, 10 g. of ether and 1 ml. of 10 per cent. ammonia were added and the mixture was shaken vigorously for 2 minutes. After separation, 10·5 g. of the ether-chloroform layer (70 per cent. of the amount added) was weighed, and almost completely evaporated on a water-bath. The evaporation was completed after the

addition of 1 ml. of ethanol (90 per cent.). The residue was then treated as for nux vomica seeds.

Dry Extract of Nux Vomica. About 0·05 g., accurately weighed, was dissolved in 5 ml. of water, acidified with a drop of 10 per cent. sulphuric acid, by warming on a water-bath. When cool, 5 g. of chloroform, 10 g. of ether and 1 ml. of 10 per cent. ammonia were added to the solution which was shaken vigorously for 2 minutes. After separation 10·5 g. of ether-chloroform layer (70 per cent. of the amount taken) was weighed off and evaporated almost completely on a water-bath. 1 ml. of ethanol (90 per cent.) was added and the solution was evaporated to dryness. The residue was then treated as for nux vomica seeds.

TABLE III

DETERMINATION OF THE ALKALOIDS OF NUX VOMICA BY ION-EXCHANGE (ANTIMONY ELECTRODE)

	Experiment No.	Amount used g.	Volume of acid used ml.	Alkaloidal Content per cent.	Alkaloidal content by Czechoslovak Pharmacopœia I method per cent.
Seeds	1	0·3017	1·20	2·05	
	2	0·3303	1·24	1·94	2·01
	3	0·3138	1·23	2·03	
	4	0·2101	0·86	2·12	
Tincture	1	2·4580	1·20	0·252	
	2	2·5621	1·24	0·250	0·250
	3	2·0349	0·97	0·247	
	4	2·2846	1·07	0·242	
Dry extract	1	0·0504	1·38	14·16	
	2	0·0486	1·32	14·05	14·24
	3	0·0529	1·50	14·21	
	4	0·0529	1·45	14·18	

1 ml. of 0·01N hydrochloric acid is equivalent to 0·003642 g. of alkaloids. Factor for acid used = 0·9939.

Belladonna Herb. About 2 g. of powdered herb accurately weighed was shaken vigorously with 3 ml. of 5 per cent. ammonia solution and 25 g. of ether. 5 ml. of water was then added and the mixture was vigorously shaken for 20 minutes. When separated 15 g. of the ether layer (equivalent to 60 per cent. of quantity of leaf) was weighed out and almost completely evaporated. 2 ml. of ethanol (90 per cent.) was added and the solution was evaporated to dryness. The residue was rubbed down with 2 ml. of 0·2 per cent. sulphuric acid and then exactly 10 ml. of water was added and the mixture triturated again. 10 ml. of the resulting solution (equivalent to 50 per cent. of the quantity of herb) was pipetted off and diluted with 10 ml. of ethanol and passed through the ion-exchange column. The eluate was diluted with 25 ml. of water and titrated against 0·01N acid.

Tincture of Belladonna. 25 g. of tincture was evaporated to a weight of 2·7 to 3·1 g. When cool, sufficient water was added to make the weight up to 10 g. and 8 g. of this solution was then filtered (i.e., equivalent to 20 g. of tincture) through cotton wool into a stoppered 50-ml. glass vessel. 15 g. of ether and 1 ml. of 10 per cent. ammonia were added

and the mixture was shaken vigorously for 2 minutes. After separation 9 g. of the ether solution (equivalent to 12 g. of tincture) was quickly weighed off and evaporated to dryness. The residue was then treated as for belladonna herb, the volume of solution used being equivalent to 10 g. of tincture.

Dry Extract of Belladonna. Approximately 0·5 g., accurately weighed, was dissolved in 5 ml. of water acidified with 1 drop of 10 per cent. sulphuric acid by warming on a water-bath. When cool, 20 g. of ether and 1 ml. of 10 per cent ammonia solution were added and the mixture was shaken vigorously for 5 minutes. After separation, 12 g. of the ethereal layer was evaporated to dryness as directed for belladonna herb. The 10 ml. of solution used are equivalent to 50 per cent. of the weight of extract used.

TABLE IV

DETERMINATION OF THE ALKALOIDS OF BELLADONNA BY ION-EXCHANGE (ANTIMONY ELECTRODE)

	Experiment No.	Amount used g.	Volume of acid used ml.	Alkaloidal Content per cent.	Alkaloidal content by Czechoslovak Pharmacopœia I method per cent.
Herb	1	2·0000	1·08	0·310	0·311
	2	2·0040	1·10	0·316	
	3	2·2265	1·21	0·316	
	4	1·9984	1·07	0·309	
Tincture	1	25·0	1·45	0·0417	0·0421
	2	25·0	1·45	0·0417	
	3	25·0	1·48	0·0425	
	4	25·0	1·50	0·0431	
Dry extract	1	0·5012	1·63	1·87	1·84
	2	0·4771	1·58	1·90	
	3	0·4118	1·40	1·86	
	4	0·4536	1·47	1·86	

1 ml. of 0·01N hydrochloric acid is equivalent to 0·002892 g. of alkaloids. Factor for acid used = 0·9939.

Hyoscyamus Herb. 20 g. of powdered herb was shaken vigorously with 100 g. of ether and 8 g. of 10 per cent. ammonia for at least 30 minutes and set aside for 15 minutes. The mixture was then filtered through cotton wool into a separating funnel which contained 10 ml. of water. This was carefully done so that the sediment in the ethereal solution was disturbed as little as possible. The new mixture was then shaken and, after separation, the aqueous layer was run off. 60 g. of the ethereal layer was weighed into a 150-ml. flask (equivalent to 60 per cent. of the quantity of herb used) and reduced in volume, by distillation, to approximately 10 ml. The flask was rinsed out with two quantities, each of 10 ml. of ether, the washings being added to the solution, and the whole evaporated almost to dryness. 8 ml. of ethanol (90 per cent.) was then added and the solution evaporated to dryness. The residue was warmed with 4 ml. of 0·1 per cent. sulphuric acid and thoroughly mixed. The mixture was made up to 12 g., stirred again, and, if it was turbid, filtered through cotton wool. 10 g. of the solution

(equivalent to 50 per cent. of the weight of herb) was diluted with 10 ml. of ethanol (90 per cent.), passed through the ion-exchange column, diluted further with 25 ml. of water and titrated against 0·01N acid.

Dry Extract of Hyoscyamus. About 2 g. of extract was dissolved by warming with 10 ml. of water acidified with 2 drops of 10 per cent. sulphuric acid. After cooling, 20 g. of ether and 1 ml. of 10 per cent. ammonia solution were added and the mixture was shaken vigorously for 5 minutes. When it had separated, 12 g. of the ethereal layer (equivalent to 60 per cent. of extract used) was weighed and almost completely evaporated. 1 ml. of ethanol (90 per cent.) was added and the solution was evaporated to dryness. The cooled residue was triturated first with 2 ml. of 0·2 per cent. sulphuric acid and then, after adding 10 ml. of water, 10 ml. of this solution (equivalent to 50 per cent. of the extract used) was pipetted off, diluted with 10 ml. of ethanol (90 per cent.), passed through the column further, diluted with 25 ml. of water and titrated against 0·01N acid.

TABLE V

DETERMINATION OF THE ALKALOIDS OF HYOSCYAMUS BY ION-EXCHANGE (ANTIMONY ELECTRODE)

	Experiment No.	Amount used g.	Volume of acid used ml.	Alkaloidal Content per cent.	Alkaloidal content by Czechoslovak Pharmacopœia I method per cent.
Herb	1	20·0	1·44	0·0414	0·0410
	2	20·0	1·50	0·0431	
	3	20·0	1·48	0·0425	
	4	20·0	1·48	0·0425	
Dry extract	1	1·7765	1·40	0·453	0·446
	2	1·7048	1·50	0·462	
	3	1·9588	1·51	0·445	
	4	1·8366	1·46	0·457	

1 ml. of 0·01N hydrochloric acid is equivalent to 0·002892 g. of alkaloids. Factor for acid used = 0·9939.

SUMMARY

Methods for the application of ion exchange to the assay of cinchona bark, ipecacuanha root, nux vomica seeds, belladonna herb, hyoscyamus herb and their preparations are described.

REFERENCES

1. Jindra and Pohorsky, *J. Pharm. Pharmacol.*, 1950, **2**, 361.